Egyptian Journal of Bronchology

Vol. 6, No 2, December, 2012

ORIGINAL ARTICLE

SOLUBLE TRIGGERING RECEPTOR EXPRESSED ON MYELOID CELLS –1 AS DIFFERENTIAL BIOMARKER OF PLEURAL EFFUSION

Ayman H. Abd El-Zaher*, Ali M. Abd-Ella*, Hala Nagy**, Raba Y. Talab*
Chest* and Clinical Pathology** Departments, Tanta University, Gharbia, Egypt

Correspondence to: Ayman Abd El-Zaher, E-mail: aymanabdelzaher@yahoo.com

Background: *The currently available diagnostic markers for pleural effusion have a limited role. The soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) is a molecule recently reported to play an important role in the myeloid cell mediated inflammatory response, and is up regulated in the body fluid by bacterial or fungal products.*

Objective: *This study aimed to investigate the value and significances of sTREM -1 in the diagnosis of pleural effusion caused by different diseases entities.*

Subjects and methods: *Samples of pleural fluid from 50 patients, (10 with empyema, 10 with para-pneumonic effusions, 10 with tuberculous effusion, 10 with malignant effusion and 10 with transudative effusion). Patients were assessed for the level of sTREM-1 by ELISA in pleural fluid. Total and differential cell count, LDH, glucose and protein were carried out to all studied patients.*

Results: *Level of sTREM-1 was highest in empyema, followed by infectious exudates and the level of sTREM-1 were low in transudate and non-infectious exudates and there was positive correlation between sTREM-1 and (protein, total white blood cells and neutrophil % in pleural fluid) and serum LDH and negative correlation between sTREM-1 and lymphocyte % in pleural fluid).*

Conclusion: *Level of sTREM-1 was highest in empyema, followed by infectious exudates and the level of sTREM-1 were low in transudate and non-infectious exudates and there was positive correlation between sTREM-1 and (protein, total white blood cells and neutrophil % in pleural fluid) and serum LDH and negative correlation between sTREM-1 and lymphocyte % in pleural fluid.*

Keywords*: sTREM-1, PPE, tuberculous pleuritis, PE.*

INTRODUCTION

Pleural effusions are a common clinical problem. Light's criteria[1,2] have been used to differentiate transudative from exudative effusions. Exudative pleural effusions are more complex in their etiology, being caused by malignancy, bacterial infection, TB or auto-immune pleuritic disease.[3,4] A definitive diagnosis of pleural effusion requires clinical data, examination of the pleural fluid & perhaps pleural biopsy. Both diagnosis & treatment are often delayed. Several biological markers have been proposed for the differential diagnosis of these disease entities,[5] however, neither is satisfactory for clinical practice. A convenient & reliable biological marker permitting rapid & accurate diagnosis of the cause of pleural effusion would be of value.[6,7] The triggering receptor expressed on myeloid cells (TERM-1) is a recently discovered cell surface molecule that is selectively expressed on blood neutrophils & a subset of monocytes. It is a member of the immunoglobulin super-family & is up-regulated in the presence of microbial products.[8,9] The initial characterization of TREM-1 demonstrated that TERM-1 expression is up-regulated in response to lipopolysaccharide & other microbial product.[10] The aim of this work was to measure the pleural fluid concentrations of sTREM-1 in patients with different causes of pleural effusions & to explore its usefulness in differentiating between infectious & non-infectious etiology.

SUBJECTS AND METHODS

This study was conducted in Chest Department, Tanta University Hospitals, Egypt, in the period from July 2010 to February 2011. The local institutional research ethics committee approved the protocol. It included fifty patients with pleural effusions of different etiologies & they were classified according to their final diagnosis into 5 groups [(I) empyema, (II) parapneumonic, (III) tuberculous, (IV) malignant & (V) transudative effusions]; each group consists of 10 patients.

These groups were diagnosed by clinical examination & assessment of the pleural fluid for LDH, total protein, cell differential counts, culture & cytology. A *transudative pleural effusion* was defined by:

1. Clinical & radiological (X-ray, ultrasonography or CT) evidence of unilateral or bilateral pleural effusion.

2. The ratio of LDH & protein in pleural fluid to plasma being less than 0.6 & 0.5, respectively, & the level of LDH being less than two-thirds of the plasma level.

3. No significant extra-pulmonary infection.

Malignant pleural effusion was defined as malignant cells detected on cytological examination of the effusion or pleural biopsy & no evidence of obstructive pneumonia.

Tuberculous pleural effusion (TB PE) was confirmed if the patient had one of the following criteria: mycobacteria or caseating granuloma detected in pathological examination of pleural biopsy; positive culture of mycobacteria in the pleural fluid or the pleural biopsy, sputum culture positive for mycobacteria, lymphocytic predominance with adenosine deaminase level of > 40 u/L & both clinical & radiological response to anti-TB treatment.

Para-pneumonic effusion (PPE) was diagnosed if the pleural effusion was accompanied by community acquired pneumonia but the effusion was not grossly purulent, no bacteria were detected by Gram stain & culture of the pleural fluid was negative for bacteria. Empyema was defined as a grossly purulent pleural effusion, accompanied by bacteria detected by Gram stain or a culture positive for bacteria.[2]

Patients were excluded if they were pregnant, receiving immunosuppressive therapy or chemotherapy, or had two concurrent causes of their pleural effusion.

Aspirations of pleural fluid were done under aseptic technique & local anesthesia with lidocaine 2%. Pleural fluid samples were analyzed for pH, protein, pleural fluid to serum protein ratio, lactate dehydrogenase (LDH), pleural fluid to serum LDH ratio & glucose level. Also, Gram and Ziehl-Nielsen stains were done. Pleural fluid cytology was done in malignant effusion. There is no risk or complications happened to the patients during the period of the study.

Pleural fluid samples were centrifuged at 2500 rpm for 5 minutes then 0.5 to 1 ml of supernatant was withdrawn & frozen at - 70 oC, before measurement of sTREM-1. sTREM-1 concentrations were measured in pleural fluid using a sandwich enzyme-linked immunosorbent assay (ELISA) procedure using a commercial kit (Duo-Set ELISA kit: human TREM-1 catalog no. DY 1278; Quantikine R & D systems, Minneapolis, Minn, USA) according to the manufacturer's instruction. The sTREM-1 levels were expressed as pg/ml.

Statistical analysis: The means and the standard deviation were used to describe the sample. Comparison for categorical variables was done using the (analysis of variance [ANOVA] tests and Linear Correlation Coefficient by SPSS V18). Statistical significance was defined as $P < 0.05$.

Receiver Operating Characteristics (ROC) curve was used to determine the cutoff value, sensitivity and specificity,

positive predictive value (PPV), negative predictive value (NPV) and accuracy of sTREM-1.

RESULTS

Patient characteristics: Fifty patients were enrolled in this study and their clinical features are summarized in (Table 1).The characteristics of pleural effusions are illustrated in Table 2. The concentrations of LDH, protein, sTREM-1 & total leucocytic count in transudative pleural effusion (PE) were much lower than those in pleural effusions induced by other etiologies (P < 0.001), while glucose level was significantly lower in empyaemic PE when compared with other types of pleural effusion (P = 0.047).

Patients with malignant PE showed a large proportion of lymphocytes in pleural fluid. Patients with TB PE showed a marked elevation of total cell counts & a large proportion of these cells were lymphocytes, with some neutrophils. Absolute lymphocyte counts were the highest in TB PE & were significantly higher than in PE from other causes (P < 0.001). Total cell counts in empyaemic PE were the highest among the five groups (all P < 0.001). Neutrophils were the most predominant cell type & numbers of neutrophils in empyaemic PE were significantly higher than for PE to any other cause (all P < 0.001).

Levels of sTREM-1 in the pleural effusion of different etiologies: (Table 3) shows mean & standard deviation with no statistical significant difference of sTREM-1 in subtypes of transudate PF; while in comparison the five groups of patients, the level of sTREM-1 was different (P<0.001). Levels were higher in patients with empyema, PPE & TB PE than malignant PE & transudate PE (Table 2, 4) & (Fig. 1) The level of sTREM-1 was significantly higher in empyema (3245±1013.09 pg/ml) than in PPE (2286±850.41 pg/ml, P= 0.009) & levels of sTREM-1 were also higher in TB PE (1510±310.73 pg/ml, P= 0.001). The levels of sTREM-1 in malignant PE (597±180.98 pg/ml) and transudate (421±131.01 pg/ml, P= 0.96) were similar but lower. Correlation between sTREM-1 & other biochemical & cellular parameters are illustrated in (table 5).

Using the ROC curve the sTREM-1 level of > 600 pgm/ml was estimated to be the cut off value for discrimination infectious effusions (empyema, PPE & TB PE) from non-infectious effusions (malignant & transudate PE) with 0.94 accuracy, 66.7 negative predictive value, 100% positive predictive value, 87.5 sensitivity and 100% specificity (Table 6) and (Fig. 2,3).

Table 1. Patient Characteristics

Variable	Value	Variable	Value
Gender (*n*)		Malignant PE (*n*)	
Male	32	- Lung cancer	3
Female	18	- Breast cancer	2
		- Malignant effusion	3
Age, years (range)	61 (33- 85)	- Malignant mesothelioma	1
Diagnosis		- Hepatic cancer	1
- Empyema thoracis (*n*)	10	Transudate (*n*)	
- Para-pneumonic effusion	10	- Liver cirrhosis	5
- Tuberculous pleuritis	10	- Congestive HF	3
		- Chronic renal disease	2

Table 2. Levels of Various Markers in Pleural Effusion Aspirate.

	Empyema (*n*=10)	PPE (*n*=10)	TB PE (*n*=10)	Malignant PE (*n*=10)	Transudate (*n*=10)	ANOVA	
						f	p- value
LDH (U/dl)	2736.7 ± 860.2	904.6 ±383.3	1612.5 ±425.6	1020.1 ±415.2	618 ±132.3	28.1	< 0.001*
Protein (g/dl)	4.7 ± 0.74	4.1 ±0.66	4.4 ±0.65	4.04 ±0.59	2.19 ±0.46	24.3	< 0.001*
Glucose (mg/dl)	105.8 ± 22.3	139.2 ±24.9	113 ±22.01	116.2 ±16.1	117.1 ±33.5	2.6	0.047*
WBC (× 10⁹/L)	6214.5 ± 1217.7	2784.9 ±3060.9	3121.6 ± 1077.03	1885.6 ±1015.15	854.1 ±306.2	15.4	< 0.001*
Neutrophil (%)	77.1 ± 9.04	64.1 ±9.4	18 ±4.6	14.5 ±3.02	21.8 ±5.2	185.9	< 0.001*
Lymphocyte (%)	21.5 ± 5.7	17.9 ±3.1	82.8 ±6.6	47.9 ±9.03	47.4 ±7.89	147.2	< 0.001*
sTREM-1 (pg/ml)	3245 ± 1013.09	2286 ±850.41	1510 ±310.73	597 ±180.98	421 ±131.01	36.7	< 0.001*

*Significant

Table 3. Pleural Fluid sTREM-1 in Subtypes of Transudative PE.

sTREM-1 pgm/ml	Transudate PF						ANOVA	
	Range			Mean	±	SD	F	P-value
Cardiac	400	-	680	543.33	±	140.119		
Renal	300	-	800	550	±	353.553	0.153	0.861
Hepatic	450	-	850	622.5	±	174.619		

Table 4. Pleural Fluid sTREM-1 in all Studied Groups

Groups	sTREM-1 (pgm/ml)						ANOVA	
	Range			Mean	±	SD	F	P-value
Empyema	1950	±	5500	3245	±	1013.095		
PPE	1100	±	3600	2286	±	850.414		
TB PF	1100	±	2000	1510	±	310.734	36.788	< 0.001*
Malignant PE	300	±	850	597	±	180.988		
Transudative PE	230	±	600	421	±	131.017		

Tukey's test

	Group I	Group II	Group III	Group IV
Group II	0.009*			
Group III	< 0.001*	0.053		
Group IV	< 0.001*	< 0.001*	0.015*	
Group V	< 0.001*	< 0.001*	0.002*	0.968

*Significant

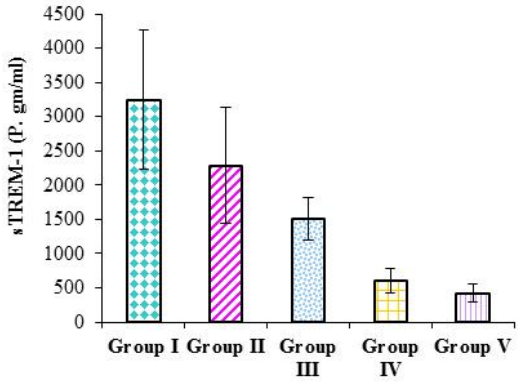

Fig 1. sTREM-1 in all Studied Groups

Table 5. Correlation Between Pleural Fluid sTREM-1 & Other Pleural Fluid Parameters.

	sTREM-1 pgm/ml	
	R	**P-value**
LDH (u/l)	0.548	< 0.001*
Glucose (mg/l)	0.003	0.984
WBCs (× 10^9/L)	0.536	< 0.001*
Neutrophils (%)	0.767	< 0.001*
Lymphocyte (%)	-0.438	< 0.001*
Protein (gm/l)	0.591	< 0.001*

*Significant

Table 6. ROC Curve Between Groups (I+II+III) and Groups (IV+V).

ROC Curve between positive (I+II+III) and negative (IV+V)					
Cutoff	**Sens.**	**Spec.**	**PPV**	**NPV**	**Accuracy**
> 600	87.5	100.0	100.0	66.7	0.945

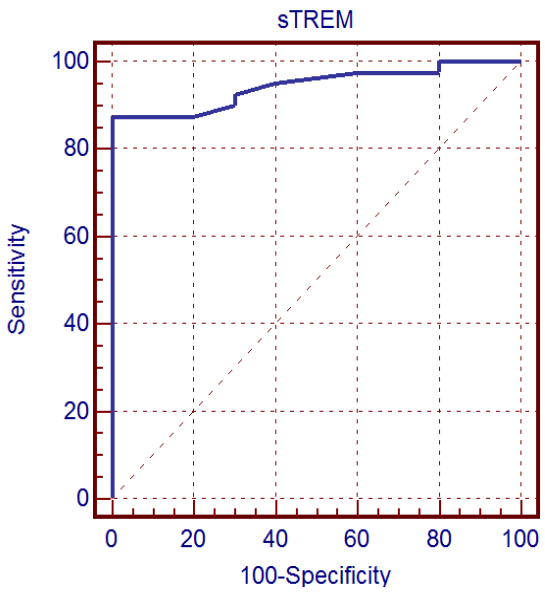

Fig 2. ROC Curve for Specificity and Sensitivity Between Groups (I+II+III) and Groups (IV+V)

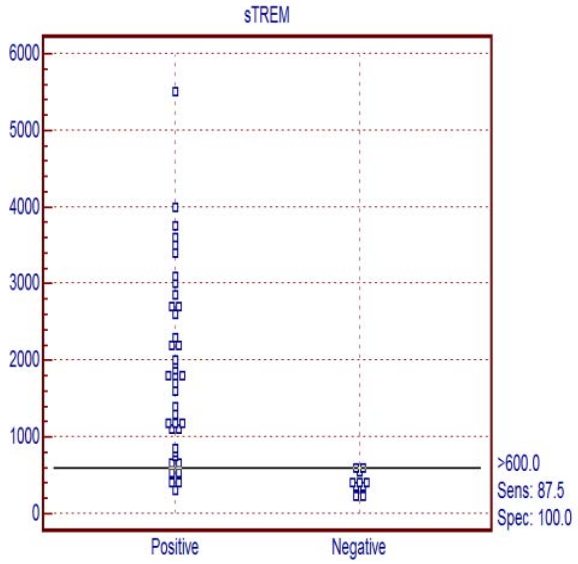

Fig 3. ROC Curve Between Groups (I+II+III) and Groups (IV+V)

DISCUSSION

A number of biochemical markers of bacterial infection have been identified, though the validity of their measurement in pleural fluid remains unclear.[11]

The development of PE is often associated with an increase of inflammatory cells in the pleural space. PE caused by diverse disease entities usually presents with a predominance of certain types of leukocytes. Bacterial pleural effusions, including empyema & PPE are typically associated with an influx of neutrophils, whereas tuberculous & malignant pleural effusions are rich in lymphocytes. [12]

TREM-1, a receptor of the immunoglobulin super-family, amplifies the inflammatory response through its over-expression & subsequent activation of neutrophils & monocytes / macrophages in response to microbial products.[11]

The aim of this study was to explore the presence of sTREM-1 in PE & its diagnostic value for differentiating infectious PE from those with PEs of other etiologies. Determining the etiology of PEs requires thoracentesis & analysis of a sample of the pleural fluid. However, a definite diagnosis may be difficult to make as the test results are often non-specific & results of microbiological tests may take days or weeks to be completed; so, a rapid & accurate test to aid clinicians is needed.[13]

Our study confirms that sTREM-1 expression is elevated in inflammatory responses to microorganisms where it is involved in the amplification of inflammation. Its activation can stimulate the release of cytokines & chemokines & induce pathogen clearance including de-granulation of neutrophils.

In the present study the concentration of sTREM-1 was highest in PE due to bacterial infection (empyema, PPE & TB) & lowest in non-infectious PEs (malignant & transudate); thus, this marker might have a role in differentiating pleural effusion formation due to bacterial & non-bacterial infection.

In accordance with our results, Phua et al. in 2006[14] found that serum levels were elevated in pneumonia. Similar results were found by Liu et al. in 2007[15] who reported that concentrations of sTREM-1 were significantly higher in infectious pleural effusions than in transudate. Among infectious effusions, the sTREM-1 levels were significantly higher in PPE than in tuberculous effusions.[16]

Huang et al. in 2008[12] reported that the concentrations of sTREM-1 in bacterial PE were significantly higher than those in malignant, tuberculous & transudative groups.

Bishara et al. in 2009[17] found that the mean levels of sTREM-1 were significantly higher in empyema than in post-thoracotomy PE & effusions of other etiologies.

In our study there was positive correlation between sTREM-1 & pleural fluid protein, WBCs, neutrophils %, and serum LDH. While, there was negative correlation between sTREM-1 & lymphocytes %.

In the present study, receiver-operating characteristic (ROC) curve analysis showed that pleural fluid sTREM-1 had a sensitivity of 87.5% and a specificity of 100% for differentiation infectious and non-infectious pleural effusion an optimal cutoff value of >600 pg/ml. The area under curve (AUC) was 0.945. Huang et al. in 2008[11] reported a pleural fluid sTREM-1 cutoff value of 768.1 pg/ml having a sensitivity of 86% and a specificity of 93% for differentiating bacterial pleural effusion from pleural effusions due to other etiologies. The AUC was 0.93, with a likelihood ratio of 2.60. Huang et al. in 2008[11] reported a pleural fluid sTREM-1 cutoff value of 768.1 pg/ml having a sensitivity of 86% and a specificity of 93% for differentiating bacterial pleural effusion from pleural effusions due to other etiologies. The AUC was 0.93, with a likelihood ratio of 2.60. Bishara et al. in 2009[17] reported a cutoff value of 114 pg/ml for pleural sTREM-1 achieved a sensitivity of 94% and a specificity of 93% in differentiating empyema from pleural effusions of other etiologies. Also, Chan M.C. et al. in 2007[13] reported pleural sTREM-1 at a cutoff value of 374 pg/ml yielded a sensitivity of 93.8%, a specificity of 90.9%, and an AUC of 0.93 in discriminating bacterial pleural infection from tuberculous pleuritis.

CONCLUSION

In conclusion, measurement of level of sTREM-1 in pleural fluid is helpful in differentiation between infectious pleural effusions (empyaemic, PPE & tuberculosis) & non-infectious pleural effusions (malignant & transudate). Our results suggest that detection of pleural sTREM-1 may improve the ability of clinician to differentiate PE patients with bacterial pleural infection from those with PE due to other causes. Pleural fluid sTREM-1 concentrations above 600 pg/ml are highly suggestive of infectious pleural effusions.

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